

Impact of the Biological Control Agent *Hydrellia pakistanae* (Diptera: Ephydriidae) on the Submersed Aquatic Weed *Hydrilla verticillata* (Hydrocharitaceae)

G. S. Wheeler and T. D. Center

USDA-ARS Invasive Plant Research Laboratory/Courtesy Associate Professor, University of Florida,
3205 College Avenue, Ft. Lauderdale, Florida 33314

Received July 25, 2000; accepted February 6, 2001

A series of studies evaluating the impact, dynamics, and distribution of the established biological control agent *Hydrellia pakistanae* (Diptera: Ephydriidae) on *Hydrilla verticillata* (Hydrocharitaceae) was conducted. The studies included establishment of fly damage thresholds with artificial infestations of flies in tanks, intensive monthly field monitoring of fly densities and damage at one site, and seasonal monitoring of fly densities in six regions in Florida. The results of tank studies indicated that fly damage approached an asymptote in the top 20 cm of the hydrilla canopy when infested with 4000 larvae/m². Above this level only a slight increase in hydrilla damage occurred as fly densities increased. Fly damage was concentrated in the top 20 cm (84.5%) and in the meristems (14.1%) of the hydrilla canopy. Hydrilla biomass was reduced with higher fly densities in the top 20 cm stratum when the plants were grown at the low fertilizer treatment. Field collections made in south Florida indicated that the hydrilla nitrogen content (fresh mass) was a major limiting factor that influenced fly densities. In the more northern regions of our studies, central and northern Florida, cold winter weather limited the seasonal distribution of the host plant and associated flies. In response to increased seasonal temperatures, fly densities increased each spring and summer. Consequently, field densities of flies and the associated damage to hydrilla populations never reached more than 15 adults/m² and 15% of the whorls damaged, respectively, about one-fifth the level estimated from cage studies that severely impact plant biomass.

Key Words: biological control of weeds; classical biological control; *Hydrilla verticillata*; *Hydrellia pakistanae*; impact of biological control; nitrogen.

Hydrilla (*Hydrilla verticillata* (L.f.) Royle) is a submersed aquatic plant that is widely distributed in the Old World (Pieterse, 1981) and has become a major aquatic weed in many areas of the southeastern United

States. Since its introduction in Florida, *H. verticillata* has extended its range west to Texas and California (Yeo and McHenry, 1977) and northeast to Maryland and Delaware (Steward *et al.*, 1984). First introduced by the aquarium trade in the 1950s, the weed has become a major threat to flood control, potable water supplies, and the biodiversity of aquatic systems. For example, 22,000 ha were infested during 1988 in Florida alone and the state spent \$7 million to control the weed in less than one-third of the affected area (Schmitz *et al.*, 1991).

Classical biological control efforts on this aquatic weed have resulted in the introduction in the southeastern United States of two fly and two weevil species from the plant's native range (Buckingham, 1994). Among these, the most widely established agent is *Hydrellia pakistanae* Deonier (Diptera: Ephydriidae), first released in 1987 (Buckingham, 1988; Center *et al.*, 1997). The larvae of *H. pakistanae* tunnel between the leaf surfaces and remove the leaf tissue, thereby reducing the total photosynthetic capacity of the plant. Each larva consumes an average of 12 leaves with a total generation time of 18 to 30 days (Baloch and Sana-Ullah, 1974).

A primary goal of this study was to compare fly densities achieved under controlled experimental conditions with those under natural field conditions and to explain these differences with abiotic and biotic factors that might influence fly densities. The factors that limit the distribution of this insect and its impact on hydrilla populations are poorly known. Possible abiotic limiting factors may include cold winter temperatures that reduce both the fly activity and the presence of its host plant hydrilla. Additionally, these relatively small (about 2 mm long) weak-flying flies may be vulnerable to sudden heavy rainfall (D. L. Deonier, personal communication), frequent events during Florida summers. Moreover, biotic mortality factors of *H. pakistanae*, such as natural enemies and variable plant quality, may be important. For example, the native parasitic

wasp *Trichopria columbiana* (Ashmead) (Hymenoptera: Diapriidae) has been reared from the immature stages of North American *Hydrellia* spp. (Deonier, 1971; Muesebeck, 1979) and from *H. pakistanae* puparia (G. S. Wheeler unpublished data); however, the frequency of this parasitism on the biological control agent *H. pakistanae* has yet to be determined. Additionally, the nitrogen content of hydrilla had a significant effect on larval mortality, growth, and development in laboratory studies (Wheeler and Center, 1996); however, its impact in field situations needs to be determined. Specifically, the goals of this study were to (1) determine the impact of a range of densities of *H. pakistanae* larvae on caged hydrilla grown in experimental tanks, (2) intensively (monthly) monitor adult and larval fly populations and the damage that they cause for 1 year at one site in south Florida, and (3) seasonally monitor fly populations at 26 sites in six regions in central and north Florida during a 2-year period. We related these fly density estimates from field collections to potential abiotic and biotic mortality factors.

METHODS AND MATERIALS

Tank Studies

Plants. Experiments were conducted in outdoor concrete tanks ($3.7 \times 6.7 \times 1$ m) at the USDA/ARS, Invasive Plant Research Laboratory in Ft. Lauderdale, Florida. Water flow in the tanks was maintained at 18.2 liters/min such that 1.4 complete tank changes occurred each day. Hydrilla tubers were sprouted (November 1993) and similarly sized germinating tubers were selected. Five tuber shoots were planted in each plastic dish pan ($35 \times 30 \times 10$ cm) containing either a low (5 g/17 kg sand) or high (15 g/17 kg sand) fertilizer level (Osmocote plus N:P:K, 18:6:12, The Scotts Co., Marysville, OH). The fertilizer was spread over the surface of the sand and then covered with a 2-cm layer of sand. Each plastic pan was completely enclosed by a cage covered with Lumite screen (Synthetic Industries, Gainesville, GA; 20.5 strands/cm²), creating a vertical rectangle $94 \times 43 \times 37$ cm. Access to each cage was through a hinged lid. The cage extended 10 cm above the water level. The flies were introduced into each cage after the hydrilla plants had completely covered the water surface. Water temperature in the tanks increased gradually from an average of $20.8 \pm 0.5^\circ\text{C}$ in December to $28.9 \pm 0.2^\circ\text{C}$ in May. Average monthly ambient temperatures during this period also increased gradually from 19.7 to 26.6°C (National Climatic Data Center, 2001).

Insects. *H. pakistanae* adults were collected (April 1994) from local field sites and transported to the laboratory where they were fed a mixture of yeast hydrolysate (4 g), sugar (7 g) in water (10 ml; Buckingham *et*

al., 1989) and allowed to oviposit on hydrilla leaves. Fly eggs and first instars were released into the cages at 15 densities (0–11,000/m²) and allowed to develop for a single generation (30 days). Cages were sealed following infestation and checked weekly during the first 2 weeks for the presence of adults and then daily during the remainder of the experiment. Adults were removed to prevent reinfestation of the plants. After all the adults emerged, the plants were harvested and divided into three sections (meristems, top 20 cm, and below 20 cm to hydrosol). The samples were assessed for fly damage by the counting of the number of damaged meristems ($n = 15$) on 10- to 15-cm lengths of shoots. Hydrilla fresh and dry biomass (after drying at 60°C for 48 h) was measured gravimetrically for each sample. To determine the percentage nitrogen for each sample ($n = 3$) leaf digests were conducted by a Kjeldahl method (Hach *et al.*, 1987) and the nitrogen content of the leaves was determined by the ammonia-selective electrode method (Greenberg *et al.*, 1992). Standard reference materials (tomato leaves: National Institute of Standards and Technology, Gaithersburg, MD) were analyzed as controls and values were adjusted for percentage recovery.

Data analysis. To determine the influence of fertilizer treatment on hydrilla plant quality, the percentage dry mass and the percentage nitrogen results were analyzed by analysis of variance (ANOVA) and means were compared with a Ryan's Q test ($P = 0.05$; SAS Institute, 1998). Hydrilla biomass data and the impact of fly density on damage were analyzed by linear and polynomial regressions.

Field Studies in South Florida

Hydrilla samples were collected monthly during 1994–1995 at one site in south Florida in the Miami Canal, of the Everglades Wildlife Management Area, Conservation Area 3. This site was chosen to monitor the fate and impact of an apparent outbreak in the *H. pakistanae* population. Samples consisted of 36 hydrilla collections (0.25–1 kg fresh mass) placed in plastic bags, stored in a cooler, and returned to the laboratory for further processing. Unlike the tank studies described previously, field collections of hydrilla were made without regard to canopy stratum. The excess water of these samples was drained from each sample for 30 min and their fresh masses (± 0.01 g) were recorded. Fly damage was recorded by the counting of the number of damaged leaves and whorls in the apical 10 cm of 36 stems. Finally, the insects associated with the samples were extracted with Berlese funnels (30.5 cm diameter \times 22.9 cm tall) equipped with a 25-W bulb for about 1 week while being maintained under ambient conditions. The insects collected from each Berlese funnel were sorted and the number of larvae and adults of *H. pakistanae* were recorded. Each sample was oven-

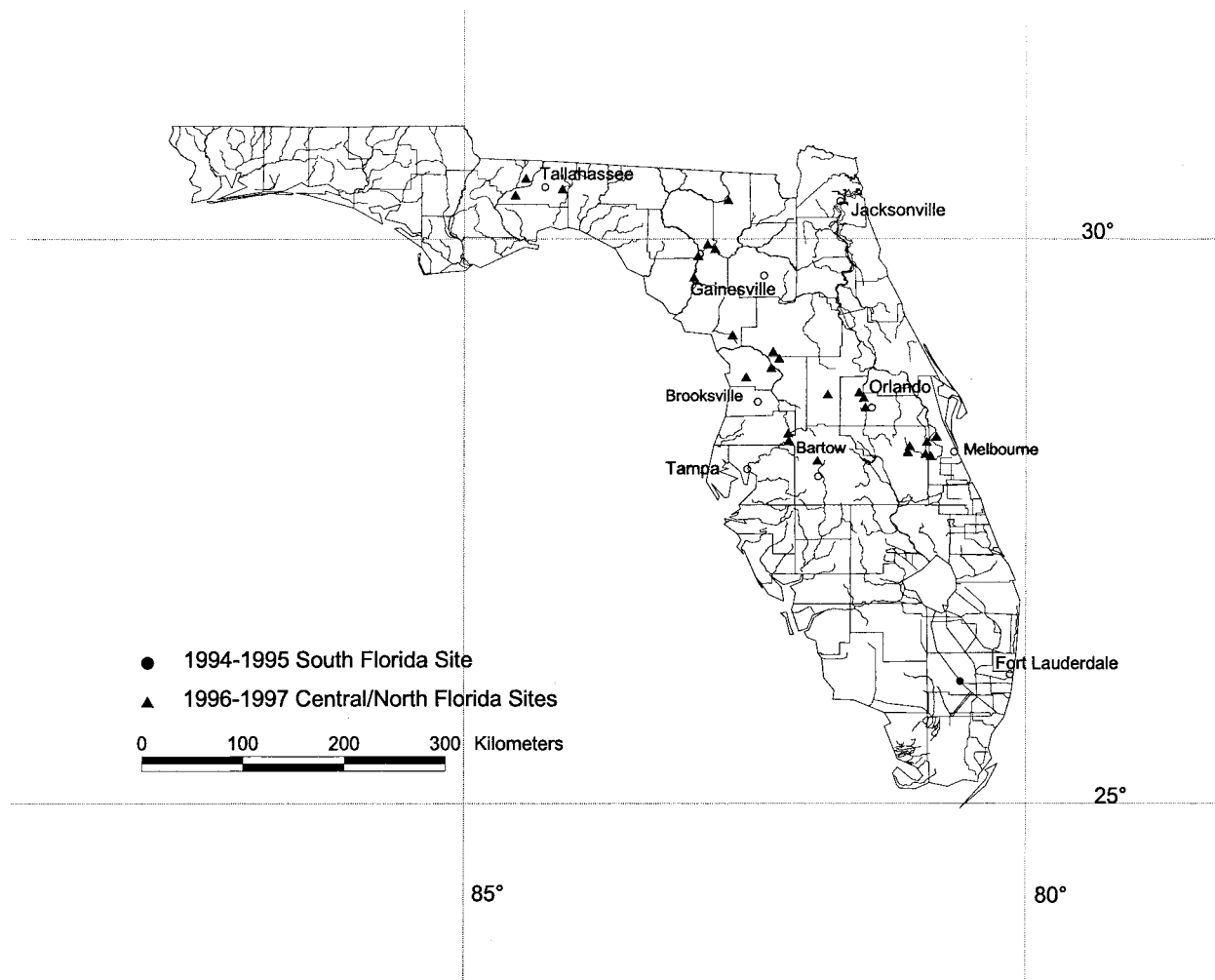


FIG. 1. Map of Florida showing collection sites of *Hydrilla verticillata* and associated *Hydrellia pakistanae* flies. A study was conducted where one site was sampled monthly during 1994–1995 in south Florida. A second study included samples collected seasonally during 1996–1997 at 26 sites in six regions of central and north Florida. Location of major cities and those that identify the regions are denoted with open circles.

dried (60°C) for 3 days and weighed. Additionally, on site, the number of adult flies was estimated by two methods: (1) gently sweeping a Styrofoam sheet (121.3 × 36.8 cm) horizontally 180° while oriented flat on the water surface/hydrilla mat ($n = 20$), and (2) counting the number of flies inside a 0.25-m² quadrat ($n = 20$) previously (1 h) placed on the hydrilla mat. These adult counts were conducted while the observer gently approached in a row boat from about 1 m distance. The different *Hydrellia* spp. could be distinguished only from the adult counts that emerged from the Berlese funnel samples. The percentages of hydrilla dry mass and nitrogen analyses were conducted as described previously. Climatic data were obtained from the South Florida Water Management District weather station (S-9) located in Conservation Area 3-A about 12 km east of the site.

To examine the changes in the percentage dry mass and nitrogen of hydrilla during the season, linear and

polynomial regressions were performed over the time period of the sample collection. To examine the relationships among plant nitrogen (fresh mass), fly densities, and leaf damage, linear regression analyses were performed.

Field studies in Central/North Florida

Hydrilla samples were collected quarterly for 2 years during 1996–1997 in six regions of Florida at 26 sites (Fig. 1). The regions are identified by the cities Tallahassee, Gainesville, Brooksville, Orlando, Bartow, and Melbourne, Florida that were near the sites. Within each region, 3–6 sites were established. Each collection consisted of three to four hydrilla samples (0.25–1 kg fresh weight) placed in plastic bags and processed by Berlese funnel extraction as described above. Following collection, each sample was placed directly into a cooler and returned to the laboratory for processing

within 48 h of collection. For nitrogen analyses, samples were digested by a modification of the aluminum block digestion procedure (Gallaher *et al.*, 1975). Nitrogen content of the digested sample was estimated by semiautomated colorimetry (Hambleton, 1977).

Climate data. Daily maximum/minimum temperature and precipitation data for 1995–1997 were obtained from the National Climatic Data Center (2001) for weather stations located in Tallahassee, Gainesville, and Orlando. Climate data for Bartow and Brooksville were obtained from the Southwest Florida Water Management District and the Melbourne data were obtained from the St. John's River Water Management District. The average daily temperature was calculated as (maximum/minimum)/2. To determine the influence of cold winter weather on hydrilla quality and *H. pakistanae* populations, we included winter data from November and December of the previous calendar year.

Data analysis. To examine the changes in the percentage dry mass and percentage nitrogen of hydrilla with regard to year, season, or region, a repeated-measures ANOVA was performed. The number of flies collected (larvae and adults) in each sample was pooled within each of the six regions and their numbers were adjusted for the fresh mass of hydrilla collected. To determine whether the density of flies changed with regard to year, season, region, or their interactions, the data were analyzed by a repeated-measures ANOVA. The relationship between fly densities and seasonal temperatures was examined with linear regression analysis.

RESULTS

Tank Studies

Plant quality. In the controlled study, where plants were fertilized at two levels and infested with a range of fly densities, the percentage dry mass of hydrilla was influenced by the fertilizer treatments ($F_{1,26} = 14.24$; $P = 0.0008$) but not by flies. Furthermore, the percentage dry mass of only the top 20 cm and the below 20 cm strata sampled were influenced by fertilizer treatment. The percentage dry mass (mean \pm SE) of the top 20 cm samples of the low fertilizer treatment was significantly greater ($12.1 \pm 0.4\%$) than that of the high fertilizer treatment ($8.8 \pm 0.4\%$). Similarly, the percentage dry mass of the low fertilizer treatment below 20 cm samples ($9.1 \pm 0.6\%$) was significantly greater ($F_{1,26} = 6.88$; $P = 0.0144$) than that of the high fertilizer treatment ($6.9 \pm 0.2\%$).

Because of these differences in hydrilla percentage dry mass and the consequent dilution of nutrients available to insect herbivores (Slansky, 1993; Wheeler and Halpern, 1999), the nitrogen results are expressed on a fresh-mass basis. Nitrogen content of the hydrilla

samples was influenced by fertilizer treatments ($F_{1,54} = 10.94$; $P = 0.0017$) and the plant strata sampled ($F_{2,54} = 7.07$; $P = 0.0019$); however, their interaction was not significant. Although, the percentage nitrogen of hydrilla from different strata did not differ within fertilizer treatments, significant or marginally significant (i.e., the top 20 cm stratum was significant at the $P = 0.1031$ level) increases were found in the meristem, the top 20 cm strata, and the below 20 cm strata from hydrilla plants grown at the high fertilizer level compared with those from the low fertilizer plants (Fig. 2).

Fly damage. The distribution of fly damage on the hydrilla plants was concentrated in the meristems and top 20 cm strata (Fig. 3). Overall, $14.1 (\pm 0.9\%)$ and $84.5 (\pm 0.9\%)$ of the damaged whorls were found in the meristems and the upper 20 cm, respectively. Moreover, the distribution of the damage was influenced by fly density. The percentage of damaged leaves decreased in the top 20 cm stratum, where it ranged from 90 to 75% of the total damage from the lowest to the highest fly densities. Conversely, the distribution of damage occurring in the meristems increased with fly densities. No change was found in the distribution of damage that occurred in the below 20 cm stratum as the fly density increased.

As fly densities increased, a greater percentage of the meristems was damaged (Fig. 4). These damaged meristems were characterized by black and necrotic leaves. These results suggest that 10–33% ($21.7 \pm 3.9\%$) of the meristems were destroyed when fly densities equaled or exceeded 7000 fly larvae/m².

The percentage of damaged hydrilla meristematic leaves also increased as the levels of flies increased (Fig. 5A). At the highest fly densities, approximately 60% of the apical leaves were damaged by fly larvae. An explanation as to why 100% damage of the meristem leaves did not occur at the highest infestation levels may be due to the rapid production of new apical leaves of the hydrilla plant.

The percentage of whorls damaged in the top 20 cm (below the meristem) of the hydrilla plants increased with increased levels of flies (Fig. 5B). Almost 70% of the whorls were damaged in cages containing approximately 4000 fly larvae/m²; however, no observable increase in damage occurred in cages with more than 6000 flies/m². This apparent asymptotic response may be the result of competition among the fly larvae for available plant material. In the cages containing these higher levels of fly larvae, the time spent searching for available food may have reduced larval consumption and survival.

The percent whorls damaged in the lower part of the hydrilla plants (below 20 cm) increased linearly with increased levels of fly larvae infestation; however, the level of damage never exceeded 20% (Fig. 5C).

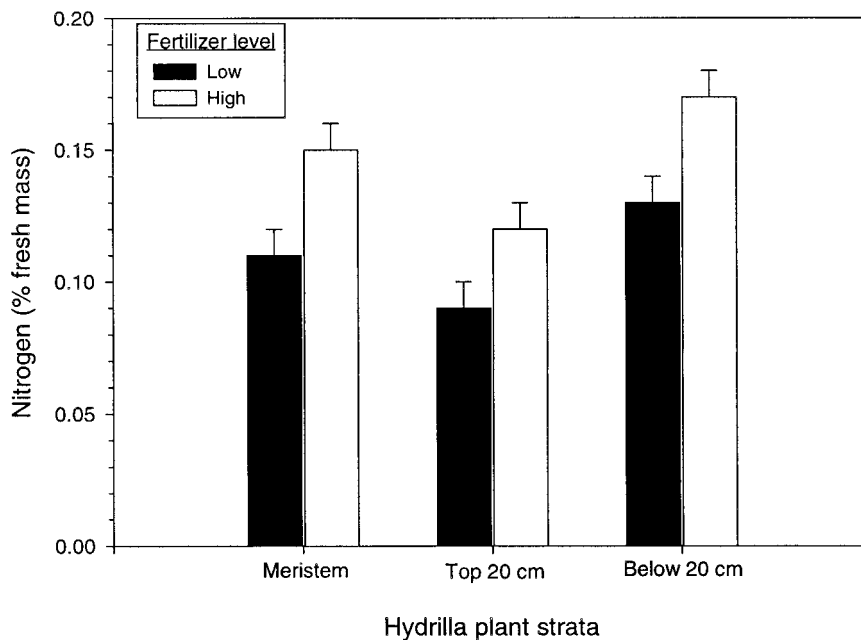


FIG. 2. Percentage nitrogen (fresh mass) from *H. verticillata* plants grown at two fertilizer levels. Different hydrilla strata, consisting of meristems, the top 20 cm, and the below 20 cm strata of the plant canopy, were collected. Although significant changes in percentage nitrogen did not occur across different strata within each fertilizer level, increased nitrogen content was found in the high fertilizer treatment in meristems ($F_{1,12} = 4.47$; $P = 0.0560$), the top 20 cm ($F_{1,18} = 2.95$; $P = 0.1031$), and the below 20 cm ($F_{1,24} = 4.49$; $P = 0.0446$) strata.

Fly impact on plant biomass. Fertilizer and fly treatments impacted hydrilla biomass only in the upper 20 cm sample ($F_{1,26} = 4.82$; $P = 0.0372$). The increased fly densities were associated with decreased hydrilla biomass (dry weight) for the low fertilizer treatment but not for the high fertilizer treatment (Fig. 6). The biomass (both dry and fresh mass) of the other hydrilla samples, below

20 cm, roots, and tubers, were not significantly influenced by either fertilizer or fly treatments.

Field Studies - South Florida

Climate data. The mean monthly temperature for the south Florida location during both 1994 and 1995

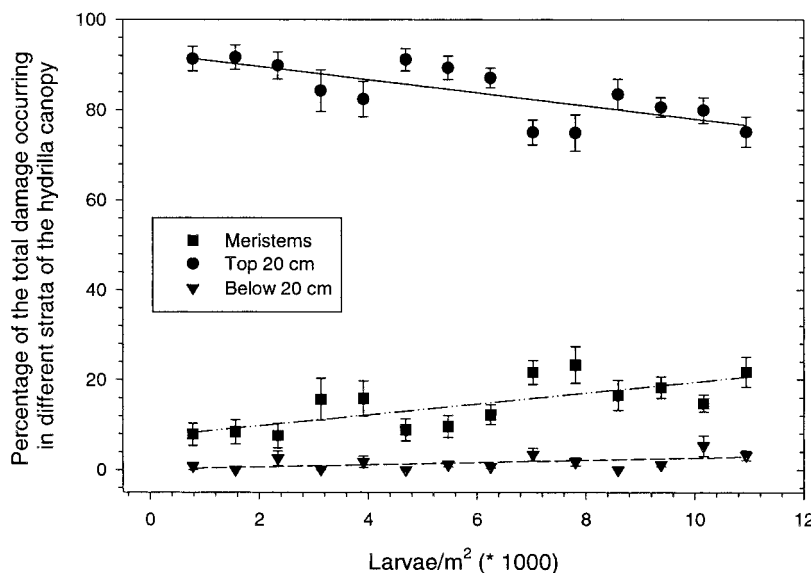


FIG. 3. Distribution of *H. pakistanae* damage in *H. verticillata* canopy strata infested at a range of fly densities. Significant decreases in the percentage of damage in the upper 20 cm ($y = 92.5 - 0.009x$; $r^2 = 0.58$; $P = 0.0016$) occurred as the fly density increased. Conversely, increases in the percentage of damage occurred in the meristems as fly density increased ($y = 7.3 + 0.008x$; $r^2 = 0.52$; $P = 0.0036$).

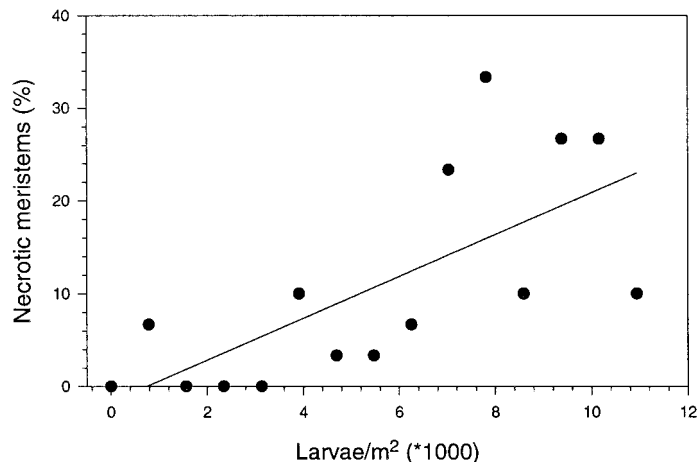


FIG. 4. Percentage of the *H. verticillata* whorls damaged by *H. pakistanae* larvae infested at different rates. A significant increase ($y = -1.7 + 2.26x$; $r^2 = 0.49$; $P = 0.0038$) in the percentage of meristems damaged was found with increased levels of flies.

ranged from 20 to 28°C. During the sampling period there was only 1 day (Feb. 9, 1995) when the mean daily temperature was less than 10°C, whereas 3 such days (Dec. 24, 25, and 26, 1994) occurred during 1994 but prior to the initiation of sampling. The mean monthly precipitation differed between 1994 and 1995 during several months. Greater rainfall occurred during most months of 1994 (April, May, July, September, November, and December) than during the same months of 1995. Severe precipitation events, or those that exceeded 25.4 mm/day, occurred 25 times during the 1994 (plus 2 months prior to the initiation of sampling) (two occurred each in May and June of 1994) and 7 times during the 1995 (six in June and one in April) sampling period. Precipitation that exceeded 50.8 mm/day occurred during the sampling period (plus 1 month prior to sampling) 6 times (July, October, November, and December) during 1994 and only once (June) during 1995. However, the occurrence of these severe rainfall events did not appear to be associated with changes in fly densities.

Plant quality. Hydrilla percentage dry mass decreased during the sampling period from 10.2 ± 0.4 to $6.2 \pm 0.2\%$ (Fig. 7). Furthermore, hydrilla percentage nitrogen ranged from $0.22 \pm 0.003\%$ fresh mass and $2.34 \pm 0.09\%$ dry mass during June to less than half those levels during February–March (Fig. 7). Percentage nitrogen significantly decreased from June 1994 through the remainder of the year and never returned to the same high level initially observed.

Hydrellia pakistanae densities. The examination of hydrilla shoots indicated that fly immatures and their damage were greatest during the early summer and these levels steadily decreased until October (Fig. 8A). Fly densities and damage increased again during No-

vember–January; however, they never reached the same level observed during June 1994.

Adult densities estimated visually by the counting of the flies inside a quadrat (0.25 m²) and by the counting of the flies that landed on a white Styrofoam board swept across the hydrilla mat similarly decreased during the sampling period (Fig. 8B). Estimates indicated that the fly densities peaked during June 1994 at 14 flies/m² and then decreased to less than 5 flies/m² during the following spring. Flies sampled by sweeping decreased from 15 flies/sweep in June 1994 to less than 5 flies/sweep the following spring.

The Berlese samples, although highly variable, con-

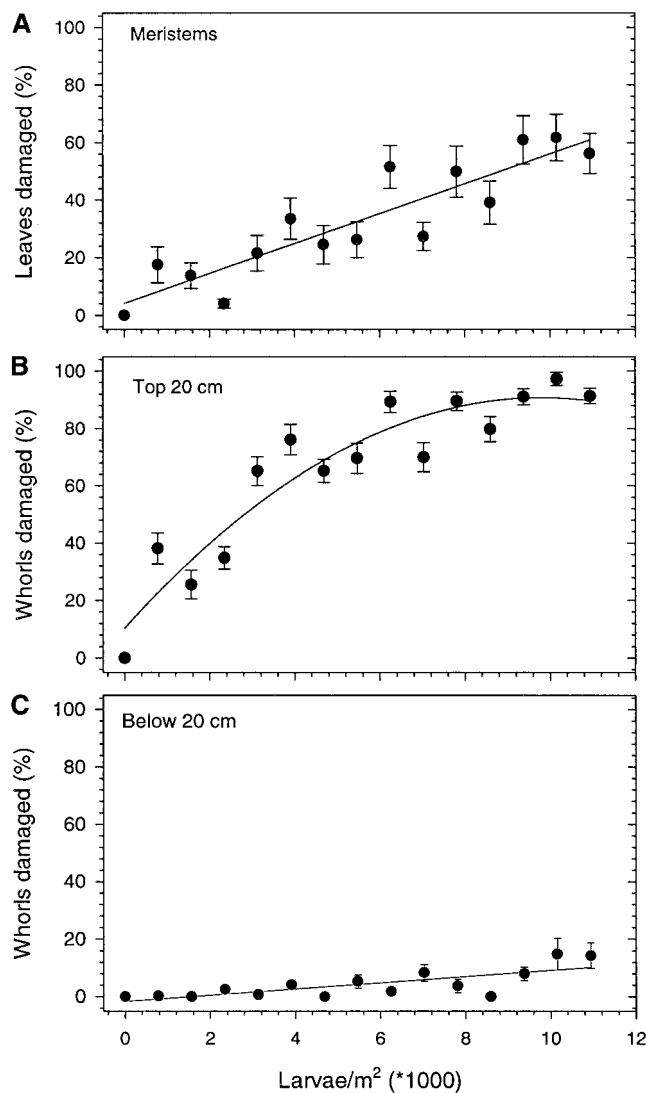


FIG. 5. Percentage of *H. verticillata* leaves (A) or whorls (B and C) damaged by larvae of *H. pakistanae* infested in cages at different rates. A significantly greater percentage of leaves (meristems; $y = 4.13 + 0.03x$, $r^2 = 0.22$, $P < 0.0001$) and whorls (B, top 20 cm $y = 10.27 + 16.44x - 0.88$, $r^2 = 0.88$, $P < 0.0001$; C, lower stratum $y = -1.66 + 0.007x$, $r^2 = 0.07$, $P < 0.0001$) were damaged with increased fly densities.

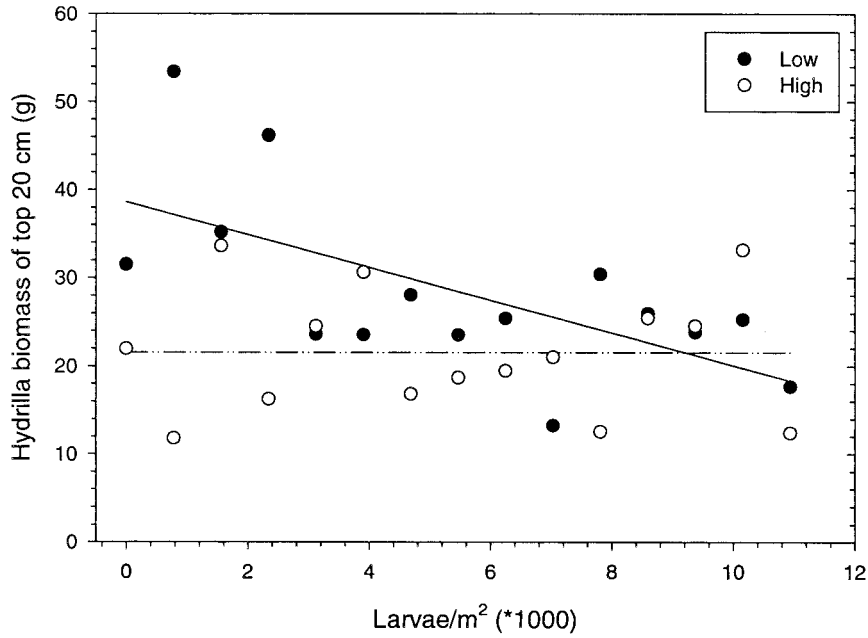


FIG. 6. Impact of *H. pakistanae* larval damage on the top 20 cm biomass of *H. verticillata* plants that were fertilized at two levels. Flies had greater impact on the plants fertilized at the low level ($y = 38.6 - 0.01x$; $r^2 = 0.40$) than on the plants fertilized at the high level ($y = 21.5 - 0.00x$; $r^2 = 0.0$).

firmed the results from the other sampling methods. A similar seasonal decline from June 1994, when densities were 0.12 flies (adults + larvae)/g fresh mass hydrilla, was observed through the remainder of the season with a slight increase during November–January (Fig. 8C).

The number of fly larvae and the amount of damage recorded from visual examinations of hydrilla stems increased significantly with increased percentage nitrogen of hydrilla (Fig. 9). At the greatest nitrogen levels about $7.5 \pm 1.26\%$ of the whorls were damaged and 0.7 ± 0.1 flies were recovered per 10 cm of stem. A

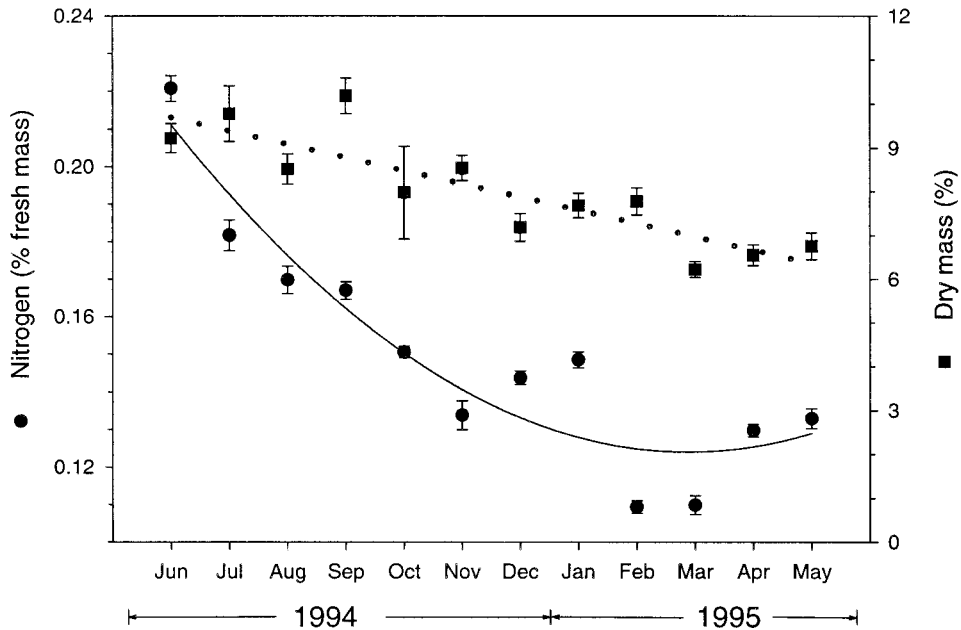


FIG. 7. Mean (\pm SE) percentage of nitrogen (fresh mass) of *H. verticillata* samples collected in the Everglades Wildlife Management Area (Miami Canal) during 1994–1995. Percentage nitrogen ($y = 0.23 - 0.02x + 0.001x^2$; $r^2 = 0.49$; $P < 0.0001$; where 'x' is the numerical month of the experiment) and percentage dry mass ($y = 28.2 - 2.7x$; $r^2 = 0.73$; $P = 0.0002$) decreased significantly during the sampling period.

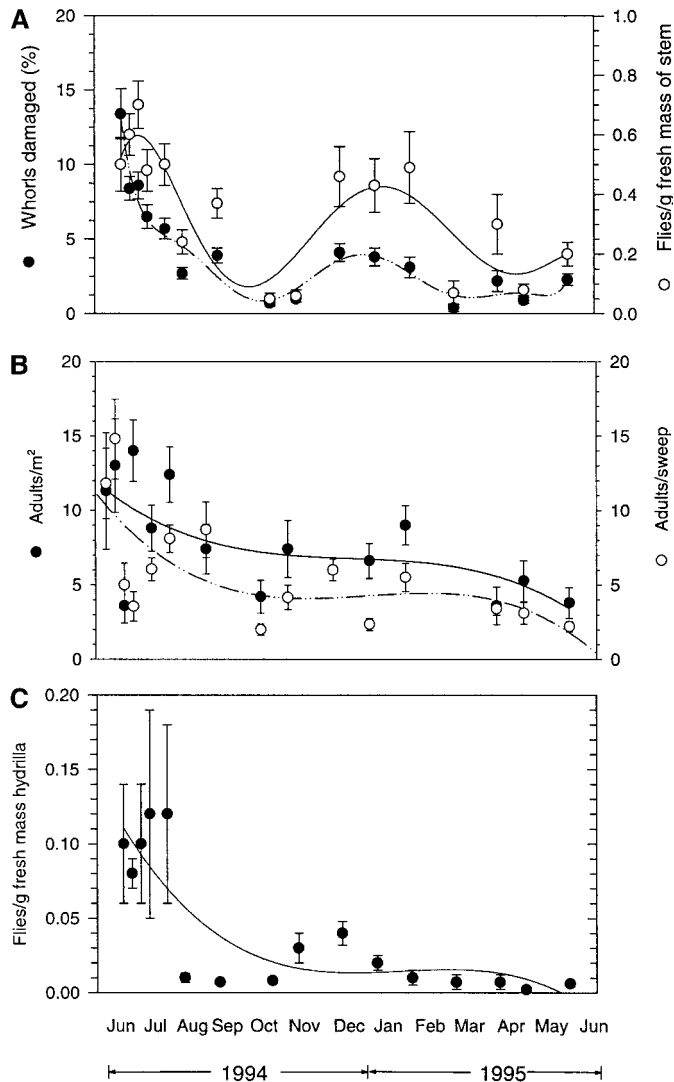


FIG. 8. Mean (\pm SE; A) percentage of *H. verticillata* whorls damaged and (A) numbers of *H. pakistanae* flies from stem inspections, (B) on-site adult quadrat and sweep collections, and (C) Berlese samples in the Everglades Wildlife Management Area (Miami Canal) during 1994–1995.

similar increase in fly densities was found in response to hydrilla percentage dry mass where the percentage whorls damaged and the mean number of flies per stem increased with increased hydrilla dry mass.

Field studies - Central/Northern Florida

Climate data. The more northern regions had generally colder temperatures and more severe winters with more days below 10°C than the more southern regions. During both years, the mean monthly temperatures reported during the winter and early spring for the more northern regions of Tallahassee (11°C) and Gainesville (14°C) were distinctly lower than those of the more southern regions (17°C; Brooksville, Orlando, and Bartow). Additionally, there was a higher fre-

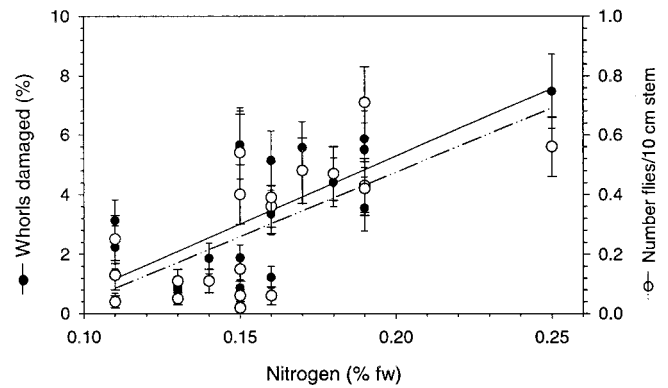


FIG. 9. Mean (\pm SE) percentage *H. verticillata* whorls damaged by *H. pakistanae* flies and mean number of flies observed by visual inspection of stems as a function of hydrilla percentage nitrogen (fresh mass) in the Everglades Wildlife Management Area (Miami Canal) during 1994–1995. Significantly more damage ($y = -4.0 + 46.8x$; $r^2 = 0.52$; $P = 0.0004$) and flies ($y = -0.4 + 4.37x$; $r^2 = 0.48$; $P = 0.0007$) were observed with increased percentage nitrogen of *H. verticillata*.

quency of severe low temperatures where the average daily temperature was equal to or less than 10°C during both seasons at the more northern regions, especially during 1996 (Table 1). Only two regions reported average daily temperatures below freezing, Tallahassee and Gainesville, which had 3 and 1 days, respectively, in 1996. None of the regions reported freezing temperatures during 1997.

Rainfall also varied by season. During 1996, peak periods of rainfall occurred during March, July, and August, whereas during 1997, the peaks occurred during April, June, and July. The most obvious difference

TABLE 1

Number of Days When the Average Daily Temperature Was ≤ 1 or 10°C and When Rainfall was ≥ 25.4 or 50.8 mm^a

Year	Location	Temperature		Rainfall	
		Days $\leq 10^\circ\text{C}$	Days $< 0^\circ\text{C}$	Days ≥ 25.4 mm	Days ≥ 50.8 mm
1996	Bartow	12	0	14	2
	Brooksville	19	0	11	2
	Gainesville	38	1	10	6
	Melbourne	13	0	13	3
	Orlando	20	0	16	3
	Tallahassee	56	3	15	2
1997	Bartow	5	0	13	5
	Brooksville	7	0	16	6
	Gainesville	16	0	14	4
	Melbourne	4	0	16	3
	Orlando	5	0	17	1
	Tallahassee	30	0	18	7

^a The climate data from November and December of the previous calendar year are included as they may influence plant and insect densities during the growing season.

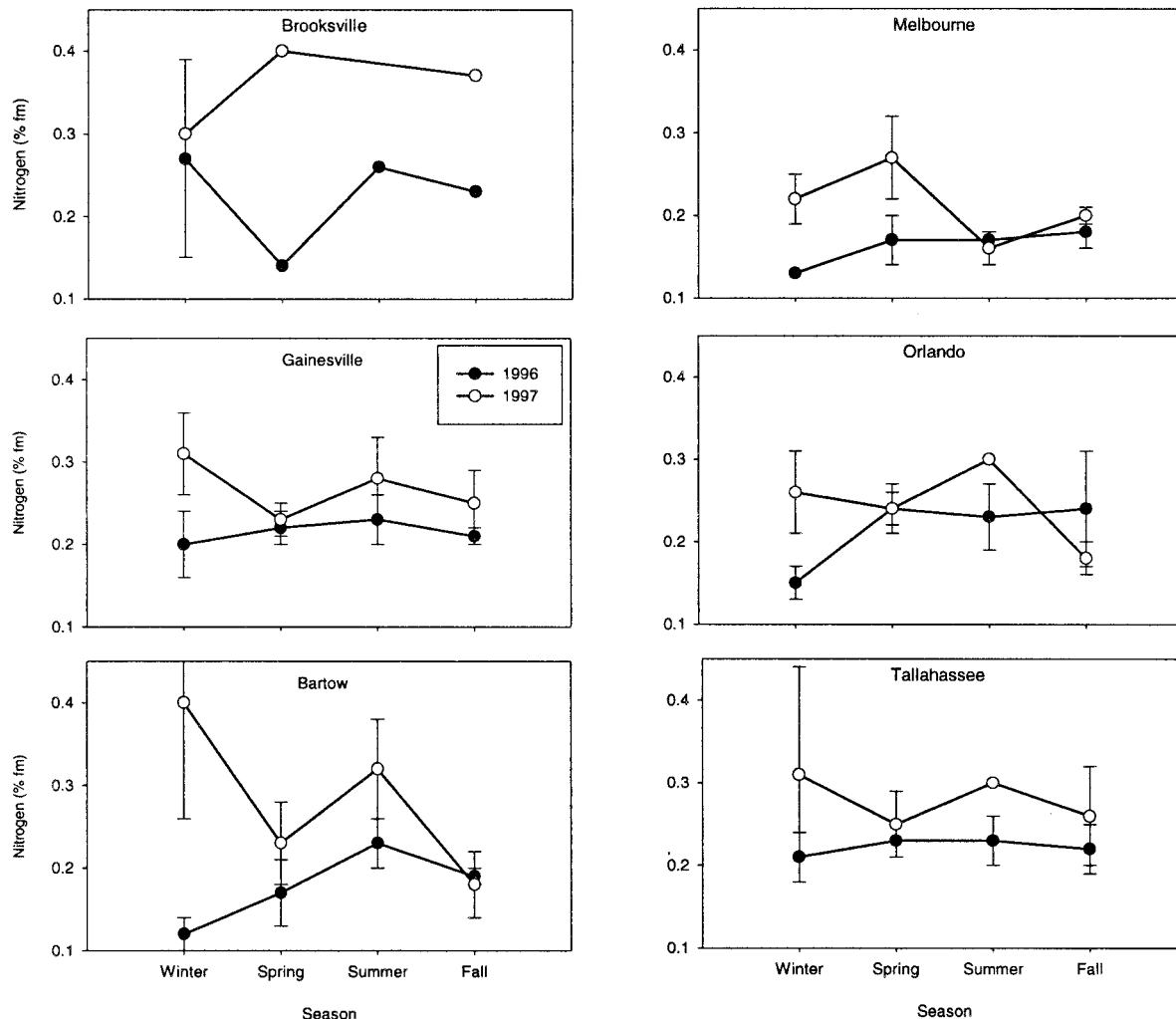


FIG. 10. Mean (\pm SE) percentage of *H. verticillata* nitrogen (fresh mass) in samples collected quarterly during 1996–1997 in six regions of Florida.

between the 2 years was the greater amount of rainfall which occurred during March of 1996 (7.3 ± 1.7 mm/day) followed by a relatively dry period during April and May. During 1997, March was relatively dry (2.2 ± 0.6 mm/day) followed by a gradual monthly increase in precipitation through June–July. However, little difference was apparent in the frequency of extreme rainfall that exceeded 25.4 or 50.8 mm/day during the 2-year sampling period in the six regions (Table 1).

Plant quality. The percentage dry mass of hydrilla was influenced by the interaction between seasons and years ($F_{3,18} = 4.88$; $P = 0.0118$). The winter hydrilla collections of 1996 had relatively low percentage dry mass ($5.6 \pm 0.5\%$), whereas samples collected during the same period of 1997 had relatively high percentage dry mass ($9.9 \pm 0.9\%$). Overall, the hydrilla from 1996 had lower percentage dry mass ($6.9 \pm 0.2\%$; $F_{1,18} = 14.26$; $P = 0.0092$) than material collected during 1997 ($8.6 \pm 0.4\%$). The regions where the hydrilla was

collected had no significant influence on percentage dry mass. Colder winter temperatures in the more northern regions generally precluded sampling of healthy hydrilla; therefore, our collections were made toward the end of winter (mid-March) after the temperatures increased and the hydrilla was found again in the water bodies.

The percentage nitrogen in hydrilla (fresh mass) differed between the 2 years of the study, during different seasons, and across different regions in which the samples were collected. Overall, percentage nitrogen was significantly ($F_{1,6} = 29.34$; $P = 0.0016$) greater during 1997 ($0.26 \pm 0.01\%$ fresh mass; $3.05 \pm 0.07\%$ dry mass) than 1996 ($0.20 \pm 0.01\%$ fresh mass; $2.88 \pm 0.05\%$ dry mass) (Fig. 10). Percentage nitrogen was lower ($F_{1,18} = 14.23$; $P = 0.0014$) during winter 1996 ($0.17 \pm 0.02\%$ fresh mass; $3.09 \pm 0.09\%$ dry mass) than during winter 1997 ($0.29 \pm 0.03\%$ fresh mass; 0.15% dry mass). Analysis of the significant year by

season by region interaction ($F_{12,18} = 3.30$; $P = 0.0111$) indicated that the highest nitrogen level was found in hydrilla collected at Bartow during winter 1996 ($0.40 \pm 0.14\%$ fresh mass; $3.51 \pm 0.13\%$ dry mass), whereas the lowest was found in the same region and season in 1997 ($0.12 \pm 0.02\%$ fresh mass; $3.00 \pm 0.11\%$ dry mass). During summer 1997, the percentage nitrogen in hydrilla was significantly ($F_{4,8} = 16.72$; $P = 0.0006$) lower at Melbourne ($0.16 \pm 0.02\%$ fresh mass; $3.32 \pm 0.36\%$ dry mass) than at the other regions (0.28 – 0.32% fresh mass; 2.99 – 4.29% dry mass) during the same year and season.

Hydrellia bilobifera and parasitoid densities. We recovered both the North American species *Hydrellia bilobifera* Cresson and the introduced biological control agent *H. pakistanae* from our hydrilla samples. As only the adult stage can be identified to species, their ratio served as our best indication of the species composition of the larvae collected. A total of 1059 *Hydrellia* spp. larvae were recovered from the Berlese samples over the 2-year period. Additionally, we recovered 185 *H. pakistanae* and 56 *H. bilobifera* adults. This species ratio suggests that about 77% of the larvae recovered from the Berlese samples were *H. pakistanae*. Individuals of *H. bilobifera* were recovered primarily during 1996 (92.9%) compared with 1997 (7.1%), during the spring (48.2%) and summer (32.1%) compared with winter (17.9%) and fall (1.8%), and in the Melbourne (44.6%), Gainesville (23.2%), and Orlando (21.4%) regions compared with the Bartow (8.9%), Tallahassee (1.8%), and Brooksville (0%) regions.

The *Hydrellia* spp. parasitoid, *Trichopria columbiana* was recovered in the Berlese samples from several sites and during several seasons. A total of 129 adult parasitoids was recovered from the Berlese samples. Thus, we estimate that approximately 9% of the *Hydrellia* spp. individuals may have been parasitized by *T. columbiana*. Parasitoids were regularly collected during all seasons (spring 31.8%, fall 30.2%, summer 19.4%, and winter 18.6%), however, their highest densities occurred during 1996 (80.0%) compared with 1997 (20.0%) and in the Orlando (63.6%) and Bartow (21.7%) regions compared with the Gainesville (9.3%), Melbourne (4.7%), Brooksville (0.8%), and Tallahassee (0%) regions.

Hydrellia pakistanae densities. Although the fly densities (per fresh mass of hydrilla) were generally low, they followed seasonal trends, being lowest in the winter and increasing to their highest levels in the summer and fall (Fig. 11). The highest fly densities were observed during 1997 in the Orlando region where an average of $0.06 (\pm 0.04)$ flies/g fresh mass of hydrilla were collected. Repeated-measures ANOVA indicated that this relatively high-density region resulted in a significant region by year interaction ($F_{5,31} = 3.05$; $P = 0.0236$). This interaction could be

explained by the relatively high fly densities in the Orlando region during 1997 being significantly greater than that of the other regions during this year ($F_{5,38} = 4.60$; $P = 0.0022$).

Unlike the previous study conducted in south Florida, regression analyses of the central and north Florida results indicated that there was no significant relationship between plant quality (percentage dry mass or nitrogen) and fly densities. Moreover, the occurrence and frequency of severe weather events in the different regions, such as cold temperatures and rainfall, did not appear to have an influence on fly densities; however, we did not sample immediately before and after such events. The only significant factor influencing fly densities in the central and north Florida regions was the mean seasonal temperature, where fly densities increased from winter through the summer with increased temperatures (Fig. 12). Regression analysis was not significant when the fall data were included.

DISCUSSION

Fly damage in the infested tank study was concentrated in the top 20 cm stratum of the hydrilla canopy. As fly densities increased and competition for suitable feeding sites became more intense, possibly scramble competition resulted in a decrease in the percentage of whorls damaged in the top 20 cm stratum while an increase occurred in the meristems. Possibly one of the greatest impacts of fly damage was the increased incidence of tips damaged, apparently by larval feeding. Despite high fly densities a decrease in hydrilla biomass was observed only in the low fertilized plants probably because we prevented the insects from multiplying during subsequent generations. If we had allowed the experiment to continue so that additional fly generations occurred, all leaves may have been damaged, resulting in a greater impact on hydrilla biomass at both fertilizer levels (Van *et al.*, 1998).

One goal of this study was to obtain an estimate of the number of flies necessary to damage nearly all the whorls of the hydrilla plant. *H. pakistanae* adults produce on average 70 eggs/female (Buckingham *et al.*, 1989), although they may produce fewer eggs (Krishnaswamy and Chacko, 1990; Baloch and Sana-Ullah, 1974). Our results indicated that approximately 4000 larvae were required during a single generation to damage 60–70% of the whorls of the hydrilla plant. Therefore, this would require the complete egg production of about 57 females. Field densities observed in this study rarely approached this level. During what was perceived as a high density in south Florida (Miami Canal) during June 1994, the hydrilla had at most 15% of the whorls damaged by flies. Our results of adult collections conducted at the same time indicated that there were about 15 adults/m² present. Assuming that half of these were females and each could produce

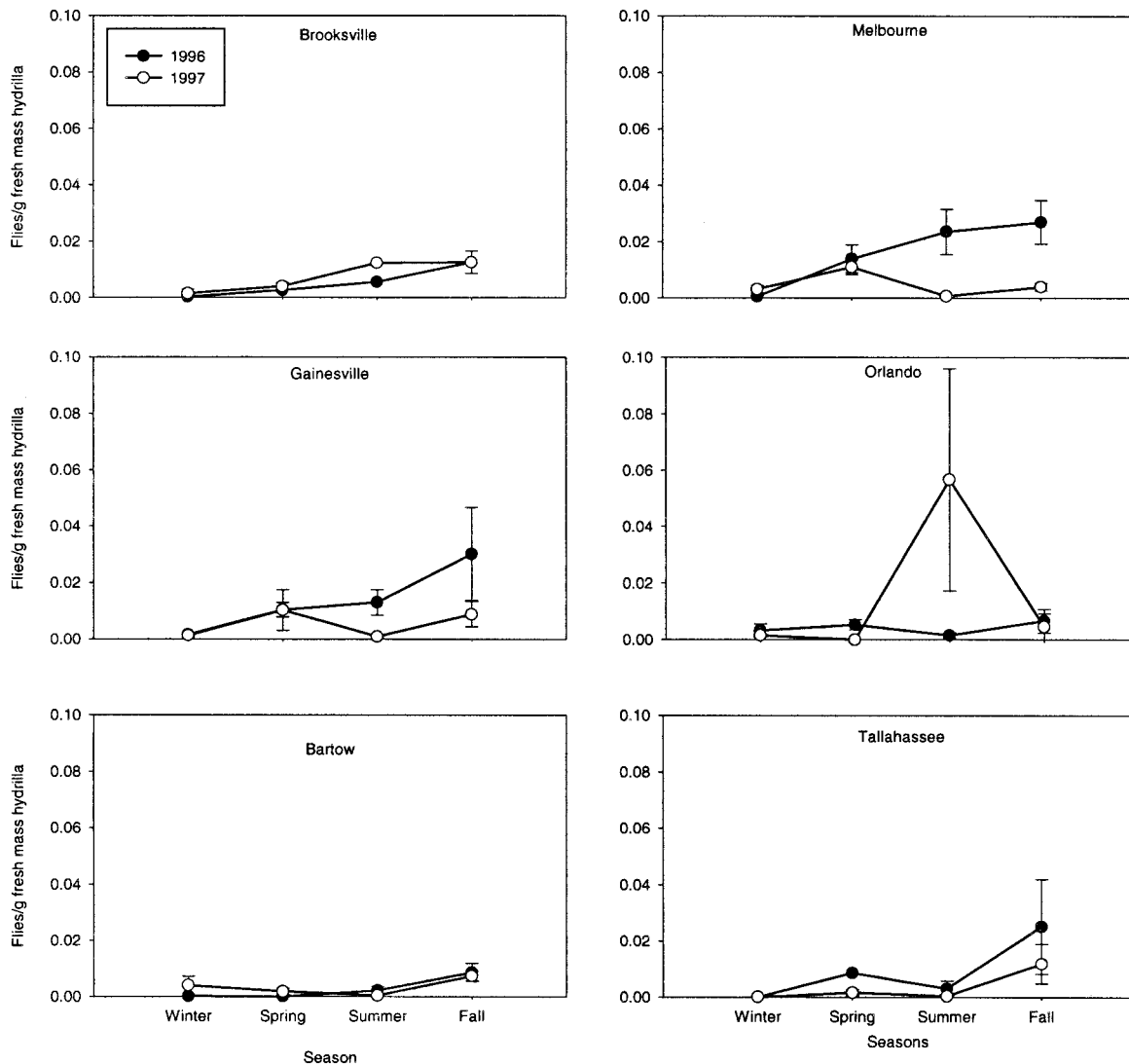


FIG. 11. Mean (\pm SE) number of *H. pakistanae* flies collected from samples collected quarterly during 1996–1997 in six regions of Florida. Samples were processed by Berlese funnel extraction for 7 days.

an average of 70 eggs, our larval population was just over 500/m², far less than that needed to cause a significant reduction in hydrilla biomass. Field collections conducted during the remainder of the year at this site and during 2 years at the other sites in central and north Florida never approached this level of flies or damage. Possibly abiotic or biotic mortality factors could explain why these flies never achieved greater densities.

These results suggest that fly densities and the damage that they cause follow cyclical patterns during the season that respond to weather conditions and possibly other factors. In south Florida where temperatures only occasionally drop below 10°C, the highest densities of flies at one site were present during the early summer and these levels declined through the fall. In the more northern regions (e.g., Gainesville and Tallahassee) where cold winters were more severe, hydrilla was generally completely submersed or present only as reproductive propagules during the winter (Schardt, 1988), resulting in a disappearance of the flies. In these northern regions fly densities reached their highest levels during the summer and fall. Severe rainfall events were also frequent during select periods of the year, especially during the spring and early summer. However, at least for south Florida (Miami Canal), these events were typically most frequent during the flies' highest densities (e.g., June–August 1994). Possibly our monthly (south Florida) or quarterly (central and north Florida) sampling frequencies were insufficient to detect the impact of severe rainfall on fly population dynamics. Considering the short and continuous generations produced by this species, very intensive sampling schedules may have to focus

hassee) where cold winters were more severe, hydrilla was generally completely submersed or present only as reproductive propagules during the winter (Schardt, 1988), resulting in a disappearance of the flies. In these northern regions fly densities reached their highest levels during the summer and fall. Severe rainfall events were also frequent during select periods of the year, especially during the spring and early summer. However, at least for south Florida (Miami Canal), these events were typically most frequent during the flies' highest densities (e.g., June–August 1994). Possibly our monthly (south Florida) or quarterly (central and north Florida) sampling frequencies were insufficient to detect the impact of severe rainfall on fly population dynamics. Considering the short and continuous generations produced by this species, very intensive sampling schedules may have to focus

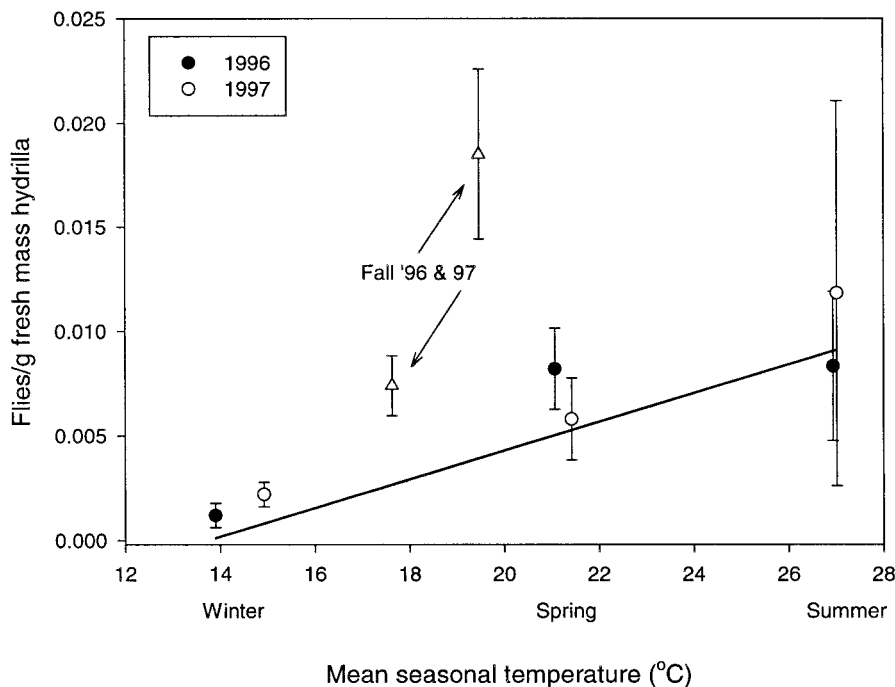


FIG. 12. Regression analysis of mean (\pm SE) number of *H. pakistanae* flies from winter through summer recovered from Berlese extractions of samples collected quarterly during 1996–1997 in six regions of Florida. Results indicate that there was a significant increase in fly densities during both years as the mean seasonal temperature increased from winter through the summer ($y = -0.009 + 0.0007x$; $r^2 = 0.12$; $P = 0.0428$). Analyses do not include the fall 1996 or 1997 data.

immediately before and after these severe events to determine impact of rainfall on fly numbers. Additionally, a combination of reduced water flow in *H. verticillata*-infested water bodies and intense summer sun may result in severe high surface-water temperatures. Many waterways in Florida have naturally low flow rates (Odum, 1957) that are undoubtedly further reduced when infested with dense infestations of submersed weeds such as *H. verticillata*. These flow rates, combined with intense sun, produce surface temperatures that may exceed 45°C (Carter *et al.*, 1991; J. Cuda, University of Florida, Gainesville, personal communication). Although flies can tolerate a range of temperatures, 100% larval mortality occurred at 36°C (Buckingham and Okrah, 1993).

Nitrogen levels in hydrilla were a significant factor influencing fly densities and damage at the south Florida site; however, this factor was not significant during the 2-year study in central and north Florida. Fly densities increased significantly with increased nitrogen content of the plants, with an increase from 0.1 to 0.25% (fresh mass) resulting in a greater percentage of the whorls damaged. These results support other studies indicating the importance of the nitrogen content of hydrilla on *H. pakistanae* performance (Wheeler and Center, 1996). The decreasing percentage of dry mass of hydrilla that occurred possibly accounted for part of the decrease in nitrogen as this and other essential nutrients became more diluted by water. As with an-

other species, *Pistia stratiotes* L. (Wheeler *et al.*, 1998), the nitrogen level of aquatic plants appear to be similar to that of other terrestrial plants when analyzed on a dry-mass basis (Lodge, 1991; Newman, 1991). However, when the relatively high water content of the plants (generally 90–95%) and its dilution of nutrients are considered, the nitrogen content on a fresh-mass basis reveals exceedingly low nitrogen levels. Nutrients expressed on this fresh-mass basis are possibly more meaningful nutritionally to herbivores in regulating their consumption and other metabolic processes (Slansky, 1993).

The nitrogen content of plants from central and north Florida generally exceeded those found in south Florida, as they ranged from 0.20% (1996 yearly average) to 0.26% (1997 yearly average), although they dropped below 0.20% several times, especially during the winters. However, the fly densities did not appear to respond to increased nitrogen levels either when it was higher 1 year than another (e.g., 1997 versus 1996) or according to regional or seasonal differences (e.g., Bartow during winter 1996). Overall, the nitrogen levels of hydrilla were generally higher in central and north Florida than in south Florida, and consequently the fly populations may have been less influenced by this factor.

Another biotic factor that may contribute to these cycles is parasitism from the diapiiid wasp *T. columbiana*. *Hydrellia* spp. larval/pupal mortality due to par-

asitism by *T. columbiana* was 9% and was present during much of the year at nearly all the regions sampled. Possibly, this is an underestimate of the level of parasitism as we recorded only the parasitoids that had completed development at the time the sample was collected or during the Berlese extraction of the sample. Possibly, a more accurate estimate of fly parasitism rates may include rearing or dissection of field-collected pupae. Although parasitism of *H. pakistanae* was not estimated in the south Florida study, this wasp species has been recovered from *Hydrellia* spp. in this area (G. S. Wheeler, unpublished data) and we suspect that similar parasitism levels will be found throughout the region.

Our results confirm those of other workers (Buckingham *et al.*, 1989) who suggest that a complex of herbivores will be needed to impact the population of this troublesome weed. Not only are additional host-specific species required but species that can complete their lifecycle in the hydrilla-infested waterbodies are needed. Despite introductions in Florida of another ephydrid fly, *H. balciunasi* (Grodowitz *et al.*, 1997), and two *Bagous* weevil species (Buckingham, 1994), *H. pakistanae* continues to be the only insect that is widely distributed for biological control of hydrilla throughout the southeastern United States. Future biological control candidates of hydrilla for the more northern ranges should be more cold tolerant or be able to recover rapidly from the winter decline of the host, whereas for southern regions candidates should be able to utilize hydrilla at a range of nitrogen levels.

ACKNOWLEDGMENTS

We are indebted to the technical assistance of Joanne Korvick (University of Florida, Ft. Lauderdale, FL) and Mark Endries, Keitha Dattilo, Nicole DeCrappeo, Rebecca Hale, and Theodora Frohne, AmeriCorps, Student Conservation Association. This manuscript benefited from the critical reviews of Jim Cuda (University of Florida, Gainesville, FL) and Mike Grodowitz (US Army Corps of Engineers, Vicksburg, MS). Nitrogen analyses were conducted by the Forage Evaluation Support Laboratory, Dept. of Agronomy, Univ. of Florida. Additional weather data were generously provided by Larry Fayard and Tabatha White, St. John's River Water Management District; South Florida Water Management District; and Joann Gilroy, Southwest Florida Water Management District. Financial support was provided by Florida Dept. of Environmental Protection and the Southwest Florida Water Management District. This is Florida Agricultural Experiment Station Journal Series No. R-07917.

REFERENCES

- Baloch, G. M., and Sana-Ullah. 1974. Insects and other organisms associated with *Hydrilla verticillata* (K.f.) L. C. (Hydrocharitaceae) in Pakistan. In "Proceedings of the 3rd International Symposium of Biological Control of Weeds" (A. J. Wapshere, Eds.), pp. 61–66. Commonw. Inst. Biol. Contr., Montpellier, France.
- Buckingham, G. R. 1988. Reunion in Florida-Hydrilla, a weevil, and a fly. *Aquatics* **10**, 19–25.
- Buckingham, G. R. 1994. Biological control of aquatic weeds. In "Pest Management in the Subtropics: Biological Control—A Florida Perspective" (D. Rosen, F. D. Bennett, and J. L. Capinera, Eds.), pp. 413–480. Intercept Ltd., Andover, UK.
- Buckingham, G. R., and Okrah, E. A. 1993. "Biological and Host Range Studies with Two Species of *Hydrellia* (Diptera: Ephydriidae) That Feed on Hydrilla." Technical Report A-93-7, U.S. Army Engineer Waterways Experiment Station, Vicksburg, MS.
- Buckingham, G. R., Okrah, E. A., and Thomas, M. C. 1989. Laboratory host range tests with *Hydrellia pakistanae* (Diptera: Ephydriidae), an agent for biological control of *Hydrilla verticillata* (Hydrocharitaceae). *Environ. Entomol.* **18**, 164–171.
- Carter, V., Rybicki, N. B., and Hamerschlag, R. 1991. Effects of submersed macrophytes on dissolved oxygen, pH and temperature under different conditions of wind, tide, and bed structure. *J. Freshw. Ecol.* **6**, 121–134.
- Center, T. D., Grodowitz, M. J., Cofrancesco, A. F., Jubinsky, G., Snoddy, E., and Freedman, J. E. 1997. Establishment of *Hydrellia pakistanae* (Diptera: Ephydriidae) for the biological control of the submersed aquatic plant *Hydrilla verticillata* (Hydrocharitaceae) in the southeastern United States. *Biol. Control* **8**, 65–73.
- Deonier, D. L. 1971. A systematic and ecological study of Nearctic *Hydrellia* (Diptera: Ephydriidae). *Smithson. Contrib. Zool.* **68**, 1–147.
- Gallaher, R. N., Weldon, C. O., and Futral, J. G. 1975. An aluminum block digester for plant and soil analysis. *Soil Sci. Soc. Am. Proc.* **39**, 803–806.
- Greenberg, A. E., Clesceri, L. S., and Eaton, A. D. 1992. "Standard Methods for the Examination of Water and Wastewater," 18th ed. American Public Health Association, Washington, DC.
- Grodowitz, M. J., Center, T. D., Cofrancesco, A. F., and Freedman, J. E. 1997. Release and establishment of *Hydrellia balciunasi* (Diptera: Ephydriidae) for the biological control of the submersed aquatic plant *Hydrilla verticillata* (Hydrocharitaceae) in the United States. *Biol. Control* **9**, 15–23.
- Hach, C. C., Bowden, B. K., Koplove, A. B., and Brayton, S. V. 1987. More powerful peroxide Kjeldahl digestion method. *J. Assoc. Off. Anal. Chem.* **70**, 787.
- Hambleton, L. G. 1977. Semiautomated method for simultaneous determination of phosphorus, calcium and crude protein in animal feeds. *J. Assoc. Off. Anal. Chem.* **60**, 845–852.
- Krishnaswamy, S., and Chacko, M. J. 1990. *Hydrellia* spp. (Diptera: Ephydriidae) attacking *Hydrilla verticillata* in South India. *Entomophaga* **35**, 211–216.
- Lodge, D. M. 1991. Herbivory on freshwater macrophytes. *Aquat. Bot.* **41**, 195–224.
- Muesebeck, C. F. W. 1979. Superfamily Proctotrupoidea. In "Catalog of Hymenoptera in America North of Mexico" (K. V. Krombein and H. D. J. Hurd, Eds.), pp. 1121–1186. Smithsonian Inst. Press, Washington, DC.
- National Climatic Data Center. 2001. National Oceanic and Atmospheric Administration. <http://www.ncdc.noaa.gov/ol/climate/climateresources.html>.
- Newman, R. M. 1991. Herbivory and detritivory on freshwater macrophytes by invertebrates: A review. *J. N. Am. Benthol. Soc.* **10**, 89–114.
- Odum, H. T. 1957. Trophic structure and productivity of Silver Springs, Florida. *Ecol. Monogr.* **27**, 55–112.
- Pieterse, A. H. 1981. *Hydrilla verticillata*—A review. *Abstr. Trop. Agric.* **7**, 9–34.
- SAS Institute. 1998. SAS/STAT User's Guide Version 7.0. SAS Institute, Cary, NC.

- Schardt, J. D. 1988. "1987 Florida Aquatic Flora Survey Report." Florida Department of Natural Resources, Tallahassee, FL.
- Schmitz, D. C., Nelson, B. V., Nall, L. E., and Schardt, J. D. 1991. "Exotic Aquatic Plants in Florida: A Historical Perspective and Review of the Present Aquatic Plant Regulation Program." Proc. Symp. on Exotic Pest Plants, National Park Service, U.S. Dept of Interior, Washington, D.C.
- Slansky, F., Jr. 1993. Nutritional ecology: The fundamental quest for nutrients. In "Caterpillars: Ecological and Evolutionary Constraints on Foraging" (N. E. Stamp and T. M. Casey, Eds.), pp. 29–91. Chapman & Hall, New York.
- Steward, K. K., Van, T. K., Carter, V., and Pieterse, A. H. 1984. Hydrilla invades Washington, D.C. and the Potomac. *Am. J. Bot.* **71**, 162–163.
- Van, T. K., Wheeler, G. S., and Center, T. D. 1998. Competitive interactions between Hydrilla (*Hydrilla verticillata*) and Vallisneria (*Vallisneria americana*) as influenced by insect herbivory. *Biol. Control* **11**, 185–192.
- Wheeler, G. S., and Center, T. D. 1996. The influence of hydrilla leaf quality on larval growth and development of the biological control agent *Hydrellia pakistanae* (Diptera: Ephydriidae). *Biol. Control* **7**, 1–9.
- Wheeler, G. S., and Halpern, M. D. 1999. Compensatory responses of *Samea multiplicalis* larvae in response to different fertilization levels of the aquatic weed *Pistia stratiotes*. *Entomol. Exp. Appl.* **91**, 205–216.
- Wheeler, G. S., Van, T. K., and Center, T. D. 1998. Herbivore adaptations to a low-nutrient food: The weed biological control specialist *Spodoptera pectinicornis* (Lepidoptera: Noctuidae) fed the floating aquatic plant *Pistia stratiotes*. *Environ. Entomol.* **27**, 993–1000.
- Yeo, R. R., and McHenry, W. H. 1977. Hydrilla, a new noxious aquatic weed in California. *Calif. Agric.* **31**, 4–5.